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EFFECT OF RECOMBINANT ERYTHROPOIETIN ON PERIPHERAL T LYMPHOCYTES

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Recombinant erythropoietin[1] has commonly been used to reduce the volume of homologous blood transfusion in patients undergoing cardiac opera-tions in Japan. We[2] have reported that recombinant erythropoietin treatment not only increased levels of circulating erythrocytes but also improved indices of cell-mediated immunity, and we[3] have suggested that it might help to ameliorate or prevent the impairment of immune function that can occur after cardiac operations. We subsequently administered recombinant erythropoietin in a dosage of 200 U/kg per day for 7 days in a patient with postoperative erythroderma associated with pancytopenia after a cardiac operation. The level of interleukin-2 production was also found to increase in association with lessening of symptoms. In the present study, we investigated the effect of recombinant erythropoietin on peripheral T cell response with the use of a reverse-transcriptase polymerase chain reaction (PCR), which has currently been acknowledged to detect cytokines in vitro in immunologic studies. Peripheral blood mononuclear cells were obtained 7 days postoperatively from 10 patients who underwent valvular operations. Five of the patients without complications were given recombinant erythropoietin in a dosage of 100 U/kg for 7 days, and the other five patients were not. Samples were prepared by the Ficoll-Conray method. They were adjusted to a concentration of 4×10^6 cells per milliliter and were incubated with 5, 10, 50, or 100 U/ml of recombinant erythropoietin for 6 hours at 37° C. Ribonucleic acid (RNA) was isolated by the RNAzol method (Biotecx Laboratories, Inc., Houston, Tex.). Cyclic deoxyribonucleic acid was prepared essentially as described by Becker, Quay, and Soukup.[4] The PCR primers used for this study were interleukin-2 primers (sense primer: 5′-TGTACAGGATGCAACTCCTGTCTT; antisense primer: 5′-GTTAGTGTTGAGATGATGCTTTGAC). PCR was performed for 35 cycles. Reverse-transcriptase PCR-amplified messenger RNA was electrophoresed in a 1.5% agarose gel and stained with ethidium bromide.[5] T cells separated from peripheral blood mononuclear cells mixed with 5 or 10 U/ml of recombinant erythropoietin did not express interleukin-2, whereas the samples mixed with 50 or 100 U/ml of recombinant erythropoietin had decreased expression regardless of whether patients received recombinant erythropoietin for 7 days after the operation. These findings suggest that, at the messenger RNA level, recombinant erythropoietin may inhibit production of interleukin-2 from T cells in proportion to the dose. These results suggest that further study will be needed to reveal the true effect of recombinant erythropoietin on cell-mediated immune responses.

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